L2: Entry 3 of 8 File: USPT Jan 9, 2001

US-PAT-NO: 6172184
DOCUMENT-IDENTIFIER: US 6172184 B1

TITLE: Hypersensitive response elicitor from Pseudomonas syringae and its use

DATE-ISSUED: January 9, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Collmer; Alan	Ithaca	NY		
Charkowski; Amy	Oakland	CA		
Alfano; James R.	Simi Valley	CA		

US-CL-CURRENT: 530/300; 435/410, 435/418, 435/71.1, 530/825, 800/295, 800/298

CLAIMS:

What is claimed:

1. An isolated hypersensitive response eliciting protein or polypeptide selected from the group consisting of (i) a protein or polypeptide comprising an amino acid sequence of SEQ. ID. No. 2, (ii) a protein or polypeptide encoded by a DNA molecule comprising a nucleotide sequence of SEQ, ID. No. 1, and (iii) a protein or polypeptide encoded by a nucleic acid molecule from a source other than Pseudomonas syringae pv. tomato which hybridizes to a DNA molecule comprising a nucleotide sequence of SEQ. ID. No. 1 under stringent conditions comprising hybridization at a temperature of about 65.degree. C. in a hybridization medium comprising about 1M NaCl.

2. An isolated protein or polypeptide according to claim 1, wherein the protein or polypeptide comprises an amino acid sequence of SEQ. ID. No. 2.

3. An isolated protein or polypeptide according to claim 1, wherein the protein or polypeptide is encoded by a nucleic acid molecule from a source other than Pseudomonas syringae pv. tomato which hybridizes to a DNA molecule comprising a nucleotide sequence of SEQ. ID No. 1 under stringent conditions comprising hybridization at a temperature of about 65.degree. C. in a hybridization medium comprising about 1M NaCl.

4. An isolated protein or polypeptide according to claim 1, wherein the protein or polypeptide is encoded by a DNA molecule comprising a nucleotide sequence of SEQ. ID. No. 1.

5. A composition comprising:

a protein or polypeptide according to claim 1 and a carrier.

6. A composition according to claim 5 further comprising an additive selected from the group consisting of fertilizer, insecticide, fungicide, nematacide, and mixtures thereof.

immunoblots with anti-HrpW antibodies used in conjunction with the Western Light chemiluminescence assay. Lanes: 4, Pel domain fragment; 5, hypersensitive response elicitor domain fragment; 6, HrpW.

FIG. 5 shows the elicitation in tobacco leaves of active tissue death indicative of the HR by cell-free preparations containing HrpW and the N-terminal fragment. The protein preparations analyzed in FIG. 4 were infiltrated into tobacco leaves, in some cases with 1.0 mM Lanthanum chloride. Leaves were photographed 48-hr later. Panels: A., P. syringae pv. syringae 61 HrpZ (0.12 .mu.g/ml); B, HrpW; C, harpin domain fragment of HrpW (0.22 .mu.g/ml); D, HrpZ+lanthanum chloride; E, HrpW+lanthanum chloride; F, Pel domain fragment of HrpW (1.40 .mu.g/ml).

DETAILED DESCRIPTION:

1 DETAILED DESCRIPTION OF THE INVENTION

2 The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 1 as follows:

```
TCCACTTCGC TGATTTTGAA ATTGGCAGAT TCATAGAAAC GTTCAGGTGT GGAAATCAGG        60

CTGAGTGCGC AGATTTCGTT GATAAGGGTG TGGTACTGGT CATTGTTGGT CATTTCAAGG       120

CCTCTGAGTG CGGTGCGGAG CAATACCAGT CTTCCTGCTG GCGTGTGCAC ACTGAGTCGC       180

AGGCATAGGC ATTTCAGTTC CTTGCGTTGG TTGGGGATAT AAAAAAAGGA ACTTTTAAAA       240

ACAGTGCAAT GAGATGCCGG CAAAACGGGA ACCGGTCGCT GCGCTTTGCC ACTCACTTCG       300

AGCAAGCTCA ACCCCAAACA TCCACATCCC TATCGAACGG ACAGCGATAC GGCCACTTGC       360

TCTGGTAAAC CCTGGAGCTG GCGTCGGTCC AATTGCCCAC TTAGCGAGGT AACGCAGCAT       420

GAGCATCGGC ATCACACCCC GGCCGCAACA GACCACCACG CCACTCGATT TTTCGGCGCT       480

AAGCGGCAAG AGTCCTCAAC CAAACACGTT CGGCGAGCAG AACACTCAGC AAGCGATCGA       540

CCCGAGTGCA CTGTTGTTCG GCAGCGACAC ACAGAAAGAC GTCAACTTCG GCACGCCCGA       600

CAGCACCGTC CAGAATCCGC AGGACGCCAG CAAGCCCAAC GACAGCCAGT CCAACATCGC       660

TAAATTGATC AGTGCATTGA TCATGTCGTT GCTGCAGATG CTCACCAACT CCAATAAAAA       720

GCAGGACACC AATCAGGAAC AGCCTGATAG CCAGGCTCCT TTCCAGAACA ACGGCGGGCT       780

CGGTACACCG TCGGCCGATA GCGGGGGCGG CGGTACACCG GATGCGACAG GTGGCGGCGG       840

CGGTGATACG CCAAGCGCAA CAGGCGGTGG CGGCGGTGAT ACTCCGACCG CAACAGGCGG       900

TGGCGGCAGC GGTGGCGGCG GCACACCCAC TGCAACAGGT GGCGGCAGCG GTGGCACACC       960

CACTGCAACA GGCGGTGGCG AGGGTGGCGT AACACCGCAA ATCACTCCGC AGTTGGCCAA      1020

CCCTAACCGT ACCTCAGGTA CTGGCTCGGT GTCGGACACC GCAGGTTCTA CCGAGCAAGC      1080

CGGCAAGATC AATGTGGTGA AAGACACCAT CAAGGTCGGC GCTGGCGAAG TCTTTGACGG      1140

CCACGGCGCA ACCTTCACTG CCGACAAATC TATGGGTAAC GGAGACCAGG GCGAAAATCA      1200

GAAGCCCATG TTCGAGCTGG CTGAAGGCGC TACGTTGAAG AATGTGAACC TGGGTGAGAA      1260

CGAGGTCGAT GGCATCCACG TGAAAGCCAA AAACGCTCAG GAAGTCACCA TTGACAACGT      1320
```

GCATGCCCAG AACGTCGGTG AAGACCTGAT TACGGTCAAA GGCGAGGGAG GCGCAGCGGT 1380

CACTAATCTG AACATCAAGA ACAGCAGTGC CAAAGGTGCA GACGACAAGG TTGTCCAGCT 1440

CAACGCCAAC ACTCACTTGA AAATCGACAA CTTCAAGGCC GACGATTTCG GCACGATGGT 1500

TCGCACCAAC GGTGGCAAGC AGTTTGATGA CATGAGCATC GAGCTGAACG GCATCGAAGC 1560

TAACCACGGC AAGTTCGCCC TGGTGAAAAG CGACAGTGAC GATCTGAAGC TGGCAACGGG 1620

CAACATCGCC ATGACCGACG TCAAACACGC CTACGATAAA ACCCAGGCAT CGACCCAACA 1680

CACCGAGCTT TGAATCCAGA CAAGTAGCTT GAAAAAAGGG GGTGGACTC 1729

3 This DNA molecule is known as the hrpW gene. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 2 as follows:

Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
1 5 10 15

Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
20 25 30

Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
35 40 45

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
50 55 60

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
65 70 75 80

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
85 90 95

Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
100 105 110

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
115 120 125

Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr
130 135 140

Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
145 150 155 160

Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly
165 170 175

Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
180 185 190

Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr
195 200 205

Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile
210 215 220

Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp
225 230 235 240

Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp
245 250 255

Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr
260 265 270

Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
275 280 285

Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
290 295 300

Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala
305 310 315 320

Val Thr An Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp
325 330 335

Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe
340 345 350

Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln
355 360 365

Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly
370 375 380

Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr
385 390 395 400

Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln
405 410 415

Ala Ser Thr Gln His Thr Glu Leu

immunoblots with anti-HrpW antibodies used in conjunction with the Western Light chemiluminescence assay. Lanes: 4, Pel domain fragment; 5, hypersensitive response elicitor domain fragment; 6, HrpW.

FIG. 5 shows the elicitation in tobacco leaves of active tissue death indicative of the HR by cell-free preparations containing HrpW and the N-terminal fragment. The protein preparations analyzed in FIG. 4 were infiltrated into tobacco leaves, in some cases with 1.0 mM Lanthanum chloride. Leaves were photographed 48-hr later. Panels: A., P. syringae pv. syringae 61 HrpZ (0.12 .mu.g/ml); B, HrpW; C, harpin domain fragment of HrpW (0.22 .mu.g/ml); D, HrpZ+lanthanum chloride; E, HrpW+lanthanum chloride; F, Pel domain fragment of HrpW (1.40 .mu.g/ml).

DETAILED DESCRIPTION:

1 DETAILED DESCRIPTION OF THE INVENTION

2 The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 1 as follows:

```
TCCACTTCGC TGATTTTGAA ATTGGCAGAT TCATAGAAAC GTTCAGGTGT GGAAATCAGG      60
CTGAGTGCGC AGATTTCGTT GATAAGGGTG TGGTACTGGT CATTGTTGGT CATTTCAAGG     120
CCTCTGAGTG CGGTGCGGAG CAATACCAGT CTTCCTGCTG GCGTGTGCAC ACTGAGTCGC     180
AGGCATAGGC ATTTCAGTTC CTTGCGTTGG TTGGGGATAT AAAAAAAGGA ACTTTTAAAA     240
ACAGTGCAAT GAGATGCCGG CAAAACGGGA ACCGGTCGCT GCGCTTTGCC ACTCACTTCG     300
AGCAAGCTCA ACCCCAAACA TCCACATCCC TATCGAACGG ACAGCGATAC GGCCACTTGC     360
TCTGGTAAAC CCTGGAGCTG GCGTCGGTCC AATTGCCCAC TTAGCGAGGT AACGCAGCAT     420
GAGCATCGGC ATCACACCCC GGCCGCAACA GACCACCACG CCACTCGATT TTTCGGCGCT     480
AAGCGGCAAG AGTCCTCAAC CAAACACGTT CGGCGAGCAG AACACTCAGC AAGCGATCGA     540
CCCGAGTGCA CTGTTGTTCG GCAGCGACAC ACAGAAAGAC GTCAACTTCG GCACGCCCGA     600
CAGCACCGTC CAGAATCCGC AGGACGCCAG CAAGCCCAAC GACAGCCAGT CCAACATCGC     660
TAAATTGATC AGTGCATTGA TCATGTCGTT GCTGCAGATG CTCACCAACT CCAATAAAAA     720
GCAGGACACC AATCAGGAAC AGCCTGATAG CCAGGCTCCT TTCCAGAACA ACGGCGGGCT     780
CGGTACACCG TCGGCCGATA GCGGGGGCGG CGGTACACCG GATGCGACAG GTGGCGGCGG     840
CGGTGATACG CCAAGCGCAA CAGGCGGTGG CGGCGGTGAT ACTCCGACCG CAACAGGCGG     900
TGGCGGCAGC GGTGGCGGCG GCACACCCAC TGCAACAGGT GGCGGCAGCG GTGGCACACC     960
CACTGCAACA GGCGGTGGCG AGGGTGGCGT AACACCGCAA ATCACTCCGC AGTTGGCCAA    1020
CCCTAACCGT ACCTCAGGTA CTGGCTCGGT GTCGGACACC GCAGGTTCTA CCGAGCAAGC    1080
CGGCAAGATC AATGTGGTGA AAGACACCAT CAAGGTCGGC GCTGGCGAAG TCTTTGACGG    1140
CCACGGCGCA ACCTTCACTG CCGACAAATC TATGGGTAAC GGAGACCAGG GCGAAAATCA    1200
GAAGCCCATG TTCGAGCTGG CTGAAGGCGC TACGTTGAAG AATGTGAACC TGGGTGAGAA    1260
CGAGGTCGAT GGCATCCACG TGAAAGCCAA AAACGCTCAG GAAGTCACCA TTGACAACGT    1320
```

```
GCATGCCCAG AACGTCGGTG AAGACCTGAT TACGGTCAAA GGCGAGGGAG GCGCAGCGGT      1380

CACTAATCTG AACATCAAGA ACAGCAGTGC CAAAGGTGCA GACGACAAGG TTGTCCAGCT      1440

CAACGCCAAC ACTCACTTGA AAATCGACAA CTTCAAGGCC GACGATTTCG GCACGATGGT      1500

TCGCACCAAC GGTGGCAAGC AGTTTGATGA CATGAGCATC GAGCTGAACG GCATCGAAGC      1560

TAACCACGGC AAGTTCGCCC TGGTGAAAAG CGACAGTGAC GATCTGAAGC TGGCAACGGG      1620

CAACATCGCC ATGACCGACG TCAAACACGC CTACGATAAA ACCCAGGCAT CGACCCAACA      1680

CACCGAGCTT TGAATCCAGA CAAGTAGCTT GAAAAAAGGG GGTGGACTC                  1729
```

3 This DNA molecule is known as the hrpW gene. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 2 as follows:

```
Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
1               5                  10                  15

Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
            20                  25                  30

Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
        35                  40                  45

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
    50                  55                  60

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
65                  70                  75                  80

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
                85                  90                  95

Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
            100                 105                 110

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
        115                 120                 125

Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr
    130                 135                 140

Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
145                 150                 155                 160
```

Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly
165 170 175

Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
180 185 190

Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr
195 200 205

Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile
210 215 220

Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp
225 230 235 240

Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp
245 250 255

Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr
260 265 270

Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
275 280 285

Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
290 295 300

Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala
305 310 315 320

Val Thr An Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp
325 330 335

Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe
340 345 350

Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln
355 360 365

Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly
370 375 380

Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr
385 390 395 400

Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln
405 410 415

Ala Ser Thr Gln His Thr Glu Leu

L2: Entry 5 of 8 File: USPT Jan 12, 1999

US-PAT-NO: 5858786
DOCUMENT-IDENTIFIER: US 5858786 A

TITLE: Pseudomonas syringae pv Syrinagae hrpZ gene

DATE-ISSUED: January 12, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Collmer; Alan	Ithaca	NY		
He; Sheng-Yang	Lexington	KY		

US-CL-CURRENT: 800/298; 435/252.3, 435/320.1, 435/325, 435/418, 435/69.1, 435/71.2, 435/874, 536/23.1, 536/23.7, 800/301

CLAIMS:

We claim:

1. An isolated gene encoding a Pseudomonas syringae protein capable of eliciting a hypersensitive response when said protein is introduced into tissue of a plant with which a Pseudomonas syringae pathogen is incompatible, under normal plant growth condictions, wherein the hypersensitive response is characterized by localized cell death in the plant tissue where the protein is introduced, the protein is glycine rich, lacks cysteine, is heat stable, lacks amino-terminal signal peptides, and is hydrophilic.

2. An isolated gene according to claim 1, wherein the protein has a molecular weight of 34.7 kDa.

3. An isolated gene according to claim 2, wherein the protein has an amino acid sequence corresponding to SEQ. ID. No. 5.

4. An isolated gene according to claim 3, wherein the DNA molecule has a nucleotide acid sequence corresponding to SEQ. ID. No. 4.

5. An isolated gene according to claim 1, wherein the protein is a protein fragment comprising a 25.1 kDa carboxyl terminal fragment.

6. An isolated gene according to claim 1, wherein the protein has repeat amino acid sequences corresponding to SEQ. ID. Nos. 1 and 2.

7. An expression system containing the gene according to claim,1.

8. An expression system according to claim 7, wherein the protein has a molecular weight of 34.7 kDa.

9. An expression system according to claim 8, wherein the protein has an amino acid sequence corresponding to SEQ. ID. No. 5.

10. An expression system according to claim 9, wherein the gene has a nucleotide sequence corresponding to SEQ. ID. No. 4.

11. An expression system according to claim 7, wherein the protein has a molecular weight of 25.1 kDa.

12. A host cell containing the gene according to claim 1, wherein the DNA molecule is heterologous to the host cell.

13. A host cell according to claim 12, wherein the protein has a molecular weight of 34.7 kDa.

14. A host cell according to claim 13, wherein the protein has an amino acid sequence corresponding to SEQ. ID. No. 5.

15. A host cell according to claim 14, wherein the gene has a nucleotide sequence corresponding to SEQ. ID. No. 4.

16. A host cell according to claim 12, wherein the protein has a molecular weight of 25.1 kDa.

17. A host cell according to claim 12, wherein the gene is in an expression system.

18. A transgenic plant containing the gene according to claim 1.

19. A transgenic plant according to claim 18, wherein the protein has a molecular weight of 34.7 kDa.

20. A transgenic plant according to claim 19, wherein the protein has an amino acid sequence corresponding to SEQ. ID. No. 5.

21. A transgenic plant according to claim 20, wherein the gene has a nucleotide sequence corresponding to SEQ. ID. No. 4.

22. A transgenic plant according to claim 18, wherein the protein has a molecular weight of 25.1 kDa.

23. A transgenic plant according to claim 18, wherein the protein has repeat amino acid sequences corresponding to SEQ. ID. Nos. 1 and 2.

24. An isolated gene according to claim 1, wherein the protein comprises the amino acid sequence Gly Gly Gly Leu Gly Thr Pro.

25. An isolated gene according to claim 1, wherein the protein comprises the amino acid sequence Gln Thr Gly Thr.

26. An isolated gene according to claim 1, wherein the isolated gene is a fragment of pHIR11.

27. An isolated nucleic acid having the nucleotide seqeunce of SEQ ID NO:3.

28. An isolated nucleic acid fragment of the nucleic acid of claim 27, said fragment having the nucleotide sequence of SEQ ID NO:6.

29. An isolated nucleic acid fragment of the nucleic acid of claim 28, said fragment having the nucleotide sequence of bases 1-648 of SEQ ID NO:6.

30. Escherichia coli DH5.alpha.(pSYH10) which is ATCC deposit no. 69317.

promoters of plant genes to develop specific transgenic plants. When the plant gene is "turned on", harpin would be expressed and the plant cell killed. Some appropriate plant gene promoters and their projected uses include genes involved in pollen development (resulting in the development of male sterile plants); genes that are expressed in response to infection by fungi, e.g. genes encoding phenylalanine ammonia lyase and chalcone synthase (the plant cell would be killed thereby limiting the progress of the fungus and making the plant resistant to fungal diseases); and genes involved in the development of senescence (to facilitate harvest, expression of hrp genes would result in defoliation).

55 Still another use of harpin within the scope of the present invention would be the use of harpin as a "target molecule" with which chemical compounds would be designed to react and thereby inactivate the bacterial harpin, which, because it is essential for disease, would provide a specific bacteriacide target.

56 Thus while we have illustrated and described the preferred embodiment of out invention, it is to be understood that this invention is capable of variation and modification, and we therefore do not wish to be limited to the precise terms set forth, but desire to avail ourselves of such changes and alterations which may be made for adapting the invention to various usages and conditions. Such variations and modifications, for example, would include the substitution of structurally similar sequences, for both the elicitor and hrpZ genes provided herein (whether derived from natural sources of synthetically manufactured), which function to yield substantially similar activities to those specifically described about. Thus, changes in sequence by the substitution, deletion, insertion or addition of nucleic acids (in the DNA sequences) or amino acids (in the peptide sequences) which do not substantially alter the function of those sequences specifically described above are deemed to be within the scope of the present invention. In addition, those fragments of the oligonucleotide sequence designated sequence No. 3 in the above sequence listing, i.e. the sequences shown as pSYH10, pSYH4, pSYH5, pSYH12, pSYH32, pSYH8, pSYH9, pSYH47, pSYH33, pSYH12, pSYH26, pSYH32 and pSYH33 are deemed to be within the scope of the present invention. Accordingly, such changes and alterations are properly intended to be within the full range of equivalents, and therefore within the purview of the following claims.

57 A listing of the nucleotide and amino acids described in the present application are as follows:

SEQUENCE LISTING
(1) GENERAL INFORMATION:
(iii) NUMBER OF SEQUENCES: 6
(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE:amino acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
GlyGlyGlyLeuGlyThrPro
(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE:amino acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
GlnThrGlyThr
(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1400 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
GATCCGGAACTCGGTCGTCCAGTTCTGATTTCTTGACGCCCCTTCATACC50
TGAGGGGGCTGCTACTTTTAGGAGGTTGTG80
ATGCAGAGTCTCAGTCTTAACAGCAGCTCGCTGCAAACC119
CCGGCAATGGCCCTTGTCCTGGTACGTCCTGAAGCCGAG158
ACGACTGGCAGTACGTCGAGCAAGGCGCTTCAGGAAGTT197
GTCGTGAAGCTGGCCGAGGAACTGATGCGCAATGGTCAA236
CTCGACGACAGCTCGCCATTGGGAAAACTGTTGGCCAAG275
TCGATGGCCGCAGATGGCAAGGCGGGCGGCGGTATTGAG314
GATGTCATCGCTGCGCTGGACAAGCTGATCCATGAAAAG353
CTCGGTGACAACTTCGGCGCGTCTGCGGACAGCGCCTCG392
GGTACCGGACAGCAGGACCTGATGACTCAGGTGCTCAAT431
GGCCTGGCCAAGTCGATGCTCGATGATCTTCTGACCAAG470
CAGGATGGCGGGACAAGCTTCTCCGAAGACGATATGCCG509
ATGCTGAACAAGATCGCGCAGTTCATGGATGACAATCCC548
GCACAGTTTCCCAAGCCGGACTCGGGCTCCTGGGTGAAC587
GAACTCAAGGAAGACAACTTCCTTGATGGCGACGAAACG626
GCTGCGTTCCGTTCGGCACTCGACATCATTGGCCAGCAA665
CTGGGTAATCAGCAGAGTGACGCTGGCAGTCTGGCAGGG704
ACGGGTGGAGGTCTGGGCACTCCGAGCAGTTTTTCCAAC743
AACTCGTCCGTGATGGGTGATCCGCTGATCGACGCCAAT782
ACCGGTCCCGGTGACAGCGGCAATACCCGTGGTGAAGCG821
GGGCAACTGATCGGCGAGCTTATCGACCGTGGCCTGCAA860
TCGGTATTGGCCGGTGGTGGACTGGGCACACCCGTAAAC899
ACCCCGCAGACCGGTACGTCGGCGAATGGCGGACAGTCC938
GCTCAGGATCTTGATCAGTTGCTGGGCGGCTTGCTGCTC977
AAGGGCCTGGAGGCAACGCTCAAGGATGCCGGGCAAACA1016
GGCACCGACGTGCAGTCGAGCGCTGCGCAAATCGCCACC1055
TTGCTGGTCAGTACGCTGCTGCAAGGCACCCGCAATCAG1094
GCTGCAGCC1103
TGACCGACAACCGCCTGACGGAGAACTCACGTGACCATTTCCCACCTTGG1153
TAATGTTAAAAGCATCTCGCCGGAACTCGGGCAGGATGTGCCACAGGGGC1203
TCGTTTCAGAACCGGCCCAGGCGGATGTCGACATCTTCACCGCTGCCACG1253
CAGCCGGACGGCGTTTCAAGTGGAGCGCCGCTTTCCGAGCATATCGCCAG1303
CGCAATTTCCGGCGGTCTGGGCGAAACCGAAAAAATGTCTCAGCAAGCGA1353
TGCGGTCGATGAAGAAAGCCTCCGGGACTGGAGACGCGCTGGATATC1400
(2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1023 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
ATGCAGAGTCTCAGTCTTAACAGCAGCTCGCTGCAAACC39
CCGGCAATGGCCCTTGTCCTGGTACGTCCTGAAGCCGAG78
ACGACTGGCAGTACGTCGAGCAAGGCGCTTCAGGAAGTT117
GTCGTGAAGCTGGCCGAGGAACTGATGCGCAATGGTCAA156
CTCGACGACAGCTCGCCATTGGGAAAACTGTTGGCCAAG195
TCGATGGCCGCAGATGGCAAGGCGGGCGGCGGTATTGAG234
GATGTCATCGCTGCGCTGGACAAGCTGATCCATGAAAAG273
CTCGGTGACAACTTCGGCGCGTCTGCGGACAGCGCCTCG312
GGTACCGGACAGCAGGACCTGATGACTCAGGTGCTCAAT351
GGCCTGGCCAAGTCGATGCTCGATGATCTTCTGACCAAG390
CAGGATGGCGGGACAAGCTTCTCCGAAGACGATATGCCG429
ATGCTGAACAAGATCGCGCAGTTCATGGATGACAATCCC468
GCACAGTTTCCCAAGCCGGACTCGGGCTCCTGGGTGAAC507
GAACTCAAGGAAGACAACTTCCTTGATGGCGACGAAACG546
GCTGCGTTCCGTTCGGCACTCGACATCATTGGCCAGCAA585

CTGGGTAATCAGCAGAGTGACGCTGGCAGTCTGGCAGGG624
ACGGGTGGAGGTCTGGGCACTCCGAGCAGTTTTTCCAAC663
AACTCGTCCGTGATGGGTGATCCGCTGATCGACGCCAAT702
ACCGGTCCCGGTGACAGCGGCAATACCCGTGGTGAAGCG741
GGGCAACTGATCGGCGAGCTTATCGACCGTGGCCTGCAA780
TCGGTATTGGCCGGTGGTGGACTGGGCACACCCGTAAAC819
ACCCCGCAGACCGGTACGTCGGCGAATGGCGGACAGTCC858
GCTCAGGATCTTGATCAGTTGCTGGGCGGCTTGCTGCTC897
AAGGGCCTGGAGGCAACGCTCAAGGATGCCGGGCAAACA936
GGCACCGACGTGCAGTCGAGCGCTGCGCAAATCGCCACC975
TTGCTGGTCAGTACGCTGCTGCAAGGCACCCGCAATCAG1014
GCTGCAGCC1023

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 341 amino acids

(B) TYPE:amino acid

(C) STRANDEDNESS:single

(D) TOPOLOGY:linear

(ii) MOLECULE TYPE:peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

MetGlnSerLeuSerLeuAsnSerSerSerLeuGlnThrProAla
51015
MetAlaLeuValLeuValArgProGluAlaGluThrThrGlySer
202530
ThrSerSerLysAlaLeuGlnGluValValValLysLeuAlaGlu
354045
GluLeuMetArgAsnGlyGlnLeuAspAspSerSerProLeuGly
505560
LysLeuLeuAlaLysSerMetAlaAlaAspGlyLysAlaGlyGly
657075
GlyIleGluAspValIleAlaAlaLeuAspLysLeuIleHisGlu
808590
LysLeuGlyAspAsnPheGlyAlaSerAlaAspSerAlaSerGly
95100105
ThrGlyGlnGlnAspLeuMetThrGlnValLeuAsnGlyLeuAla
110115120
LysSerMetLeuAspAspLeuLeuThrLysGlnAspGlyGlyThr
125130135
SerPheSerGluAspAspMetProMetLeuAsnLysIleAlaGln
140145150
PheMetAspAspAsnProAlaGlnPheProLysProAspSerGly
155160165
SerTrpValAsnGluLeuLysGluAspAsnPheLeuAspGlyAsp
170175180
GluThrAlaAlaPheArgSerAlaLeuAspIleIleGlyGlnGln
185190195
LeuGlyAsnGlnGlnSerAspAlaGlySerLeuAlaGlyThrGly
200205210
GlyGlyLeuGlyThrProSerSerPheSerAsnAsnSerSerVal
215220225
MetGlyAspProLeuIleAspAlaAsnThrGlyProGlyAspSer
230235240
GlyAsnThrArgGlyGluAlaGlyGlnLeuIleGlyGluLeuIle
245250255
AspArgGlyLeuGlnSerValLeuAlaGlyGlyGlyLeuGlyThr
260265270
ProValAsnThrProGlnThrGlyThrSerAlaAsnGlyGlyGln
275280285
SerAlaGlnAspLeuAspGlnLeuLeuGlyGlyLeuLeuLeuLys
290295300
GlyLeuGluAlaThrLeuLysAspAlaGlyGlnThrGlyThrAsp
305310315
ValGlnSerSerAlaAlaGlnIleAlaThrLeuLeuValSerThr
320325330
LeuLeuGlnGlyThrArgAsnGlnAlaAlaAla

335340
(2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 945 base pairs
(B) TYPE:nucleic acid
(C) STRANDEDNESS:single
(D) TOPOLOGY:linear
(ii) MOLECULE TYPE:DNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
GATCTTCTGACCAAGCAGGATGGCGGGACAAGCTTCTCC39
GAAGACGATATGCCGATGCTGAACAAGATCGCGCAGTTC78
ATGGATGACAATCCCGCACAGTTTCCCAAGCCGGACTCG117
GGCTCCTGGGTGAACGAACTCAAGGAAGACAACTTCCTT156
GATGGCGACGAAACGGCTGCGTTCCGTTCGGCACTCGAC195
ATCATTGGCCAGCAACTGGGTAATCAGCAGAGTGACGCT234
GGCAGTCTGGCAGGGACGGGTGGAGGTCTGGGCACTCCG273
AGCAGTTTTTCCAACAACTCGTCCGTGATGGGTGATCCG312
CTGATCGACGCCAATACCGGTCCCGGTGACAGCGGCAAT351
ACCCGTGGTGAAGCGGGGCAACTGATCGGCGAGCTTATC390
GACCGTGGCCTGCAATCGGTATTGGCCGGTGGTGGACTG429
GGCACACCCGTAAACACCCCGCAGACCGGTACGTCGGCG468
AATGGCGGACAGTCCGCTCAGGATCTTGATCAGTTGCTG507
GGCGGCTTGCTGCTCAAGGGCCTGGAGGCAACGCTCAAG546
GATGCCGGGCAAACAGGCACCGACGTGCAGTCGAGCGCT585
GCGCAAATCGCCACCTTGCTGGTCAGTACGCTGCTGCAA624
GGCACCCGCAATCAGGCTGCAGCC648
TGACCGACAACCGCCTGACGGAGAACTCACGTGACCATTTCCCACCTTGG698
TAATGTTAAAAGCATCTCGCCGGAACTCGGGCAGGATGTGCCACAGGGGC748
TCGTTTCAGAACCGGCCCAGGCGGATGTCGACATCTTCACCGCTGCCACG798
CAGCCGGACGGCGTTTCAAGTGGAGCGCCGCTTTCCGAGCATATCGCCAG848
CGCAATTTCCGGCGGTCTGGGCGAAACCGAAAAAATGTCTCAGCAAGCGA898
TGCGGTCGATGAAGAAAGCCTCCGGGACTGGAGACGCGCTGGATATC945

CLAIMS:

We claim:

1. An isolated gene encoding a Pseudomonas syringae protein capable of eliciting a hypersensitive response when said protein is introduced into tissue of a plant with which a Pseudomonas syringae pathogen is incompatible, under normal plant growth condictions, wherein the hypersensitive response is characterized by localized cell death in the plant tissue where the protein is introduced, the protein is glycine rich, lacks cysteine, is heat stable, lacks amino-terminal signal peptides, and is hydrophilic.

2. An isolated gene according to claim 1, wherein the protein has a molecular weight of 34.7 kDa.

3. An isolated gene according to claim 2, wherein the protein has an amino acid sequence corresponding to SEQ. ID. No. 5.

4. An isolated gene according to claim 3, wherein the DNA molecule has a nucleotide acid sequence corresponding to SEQ. ID. No. 4.

5. An isolated gene according to claim 1, wherein the protein is a protein fragment comprising a 25.1 kDa carboxyl terminal fragment.

6. An isolated gene according to claim 1, wherein the protein has repeat amino acid sequences corresponding to SEQ. ID. Nos. 1 and 2.

7. An expression system containing the gene according to claim,1.

8. An expression system according to claim 7, wherein the protein has a

WEST

Generate Collection | Print

L2: Entry 7 of 8 File: USPT Dec 15, 1998

US-PAT-NO: 5849868
DOCUMENT-IDENTIFIER: US 5849868 A

TITLE: Elicitor of the hypersensitive response in plants

DATE-ISSUED: December 15, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Beer; Steven V.	Ithaca	NY		
Wei; Zhong-Min	Ithaca	NY		
Bauer; David W.	Ithaca	NY		
Collmer; Alan	Ithaca	NY		
He; Sheng-Yang	Ithaca	NY		
Laby; Ron	Ithaca	NY		

US-CL-CURRENT: 530/350; 530/324, 530/326, 530/823, 530/825

CLAIMS:

We claim:

1. An isolated protein which elicits a hypersensitive response in different plant species when said protein is introduced into leaf tissue of a plant under normal plant growth condition, wherein said protein is encoded by a nucleic acid sequence which hybridizes to the nucleic acid of SEQ. ID. No. 4 under stringent conditions of 0.4 x SSC, 0.2% SDS washing at 65.degree. C. or wherein said protein is protease sensitive and heat stable at 100.degree. C. for at least one minute.

2. The isolated protein according to claim 1 which has a molecular size of 44 Kd and a pI of 4.3.

3. The isolated protein according to claim 1 which is a hypersensitive response elicitor protein from an Erwinia, Pseudomonas, or Xanthomonas pathogen.

4. The isolated peptide according to claim 1, wherein said protein is purified.

5. The isolated peptide according to claim 1, wherein said protein has no cysteine.

6. An isolated protein which elicits a hypersensitive response in different plant species when said protein is introduced into leaf tissue of a plant under normal plant growth conditions, wherein the hypersensitive response eliciting protein is from an Erwinia pathogen.

7. The isolated protein according to claim 6, wherein the Erwinia pathogen is Erwinia amylovora.

8. The isolated protein according to claim 7, wherein said protein has a molecular weight of 44 kDa as determined by SDS polyacrylamide gel

electrophoresis.

9. The isolated protein according to claim 7, wherein said protein has an amino acid sequence of SEQ. ID. No. 2.

10. The isolated protein according to claim 6, wherein the Erwinia pathogen is Erwinia chrysanthemi.

11. The isolated protein according to claim 6, wherein the Erwinia pathogen is Erwinia stewartii.

End of Result Set

L2: Entry 8 of 8 File: USPT Jan 13, 1998

US-PAT-NO: 5708139
DOCUMENT-IDENTIFIER: US 5708139 A

TITLE: Pseudomonas syringae pv syringae hrpZ gene

DATE-ISSUED: January 13, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Collmer; Alan	Ithaca	NY		
He; Sheng-Yang	Lexington	KY		

US-CL-CURRENT: 530/350; 435/874, 536/23.7

CLAIMS:

We claim:

1. An isolated Pseudomonas syringae protein capable of eliciting a hypersensitive response when said protein is introduced into tissue of a plant with which a Pseudomonas syringae pathogen is incompatible, under normal plant growth conditions, wherein the hypersensitive response is characterized by localized cell death in the plant tissue where the protein is introduced, the protein is glycine rich, lacks cysteine, is heat stable, lacks amino-terminal signal peptides, and is hydrophilic.

2. An isolated Pseudomonas syringae protein according to claim 1, wherein said protein comprises the amino acid sequence Gly Gly Gly Leu Gly Thr Pro.

3. An isolated Pseudomonas syringae protein according to claim 1, wherein said protein comprises the amino acid sequence Gln Thr Gly Thr.

4. An isolated protein according to claim 1, wherein the protein has a molecular weight of 34.7 kDa.

5. An isolated protein according to claim 4, wherein the protein has an amino acid sequence of SEQ. ID. No. 5.

6. An isolated protein according to claim 1, wherein the protein lacks tyrosine.

7. An isolated protein according to claim 1, wherein the protein has repeat amino acid sequences of SEQ. ID. Nos. 1 and 2.

8. An isolated protein according to claim 1, wherein the protein is purified.

9. An isolated protein according to claim 1, wherein the protein is recombinant.

10. An isolated protein fragment comprising a 25.1 carboxyl terminal fragment of the protein of claim 1.

11. An isolated protein fragment of the protein of claim 1 comprising amino acids 194 to 341 of SEQ ID NO:5.